

Detection (SKY)

Reagents

Avidin-Cy5

Jackson Immuno Research Lab, Cat. 003-170-083

Bovine Serum Albumin (BSA)

DAPI

Sigma Cat 18860

Formamide

Fluka BioChemika Cat 47671

HCl 1N

Mouse anti-digoxigenin

Sigma, Cat. D 8156

Sheep anti-mouse Cy5.5

Amersham, Cat. RPQ 0115

SSC 20X

Tween 20

ddH₂O

Preparation

50% FA/2X SSC

SSC 20X	30 ml
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ddH₂O 120 ml

Formamide 150 ml

Adjust pH to 7.0 using 1N HCl

Pre-warm to 45°C

1X SSC

20X SSC 25 ml

dH₂O 475 ml

Pre-warm to 45°C

4X SSC/0.1%Tween 20

SSC 20X 200 ml

dH ₂ O	799 ml
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Tween 20	1 ml
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Pre-warm to 45°C

Blocking Solution (3% BSA/4X SSC/0.1% Tween 20)

BSA	0.3 g
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4X SSC/0.1% Tween 20	10 ml
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Vortex until dissolved
Pre-warm to 37°C

Antibody Solution (1% BSA/4X SSC/0.1% Tween 20)
0.1 g BSA
10 ml 4X SSC/0.1% Tween 20
Pre-warm to 37°C

DAPI stock solution (f.c.= 0.2mg/ml)
2 mg DAPI
10 ml dH₂O
Aliquot and store at -80°C

DAPI staining solution (f.c.= 80ng/ml)
DAPI (stock solution) 40 µl
SSC 2X 100 ml
Store at 4°C in a light-tight coplin jar.

Procedure

1. Carefully remove rubber cement surrounding coverslips with forceps.
2. Wash slides in 50% formamide/2X SSC for 3 x 5 min each, shaking.
3. Wash slides in 1X SSC for 3 x 5 min, shaking.
4. Dip slides in 4X SSC/0.1% Tween 20.
5. Add 120 µl of Blocking Solution (3% BSA/4X SSC/0.1% Tween 20) to a 24 mm x 60 mm coverslip and incubate in a moist hybridization chamber at 37°C for 30 min.
6. Dip slides in 4X SSC/0.1% Tween 20 to wash off blocking solution.
7. Combine the two antibodies, mouse anti-Dig and Avidin-Cy5, into the same eppendorf tube (see note 3), and apply 120 µl of antibody solution to a 24 mm x 60 mm coverslip. Each antibody (Avidin-Cy5 and mouse anti-Dig) should be diluted 1:200 in 1% BSA/4X SSC/0.1% Tween 20. Invert the slide onto the solution. Incubate the slides in a moist hybridization chamber at 37°C for 45 min.
8. Wash slides in 4X SSC/0.1% Tween 20, 3 x 5 min, shaking.

9. Add 120 μ l of the antibody (sheep anti-mouse Cy5.5, diluted 1:200). Incubate slides in a moist hybridization chamber at 37°C for 45 min.
10. Wash slides 3 x 5 min, shaking.
11. Stain slides for 5 min in the DAPI staining solution in a light-protected coplin jar.
12. Wash slides with 2X SSC. Dehydrate slides in ethanol series of 70%, 90%, and 100%; for 3 min each, air-dry slides.
13. Apply 35 μ l of antifade solution, cover each slide with a 24 mm x 60 mm coverslip, and store in a light-protected container at 4°C until slide is imaged..

Notes

1. Exposure of slides to ambient light should be minimized during all procedures.
2. Carefully remove coverslips during all procedures to minimize scratches.
3. Spin all fluorescent dyes prior to use for 3 min at 13,000 rpm.
4. Do not let the slide dry out between washing steps.